Effect of dietary supplements in reducing probability of death for uremic crises in dogs affected by chronic kidney disease (masked RCCT)

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Abstract: Chitosan and alkalinizing agents can decrease morbidity and mortality in humans with chronic kidney disease (CKD). Whether this holds true in dog is not known. Objective of the study was to determine whether a commercial dietary supplement containing chitosan, phosphate binders, and alkalinizing agents (Renal), compared to placebo, reduces mortality rate due to uremic crises in dogs with spontaneous CKD, fed a renal diet (RD). A masked RCCT was performed including 31 azotemic dogs with spontaneous CKD. Dogs enrolled in the study were randomly allocated to receive RD plus placebo (group A; 15 dogs) or RD plus Renal (group B; 16 dogs). During a first 4-week period, all dogs were fed an RD and then randomized and clinically evaluated up to 44 weeks. The effects of dietary supplements on mortality rate due to uremic crises were assessed. At 44 weeks, compared to group A, dogs in group B had approximately 50% lower mortality rate due to uremic crises ($P = 0.015$). Dietary supplementation with chitosan, phosphate binders, and alkalinizing agents, along with an RD, is beneficial in reducing mortality rate in dogs with spontaneous CKD.

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Structured abstract

Background—Chitosan and alkalinizing agentscan decrease morbidity and mortality concentrationsin humans with chronic kidney disease (CKD). Whether this holds true in dogs is not known.

Objective—To determine whether two commercial dietary supplements containing chitosan and alkalinizing agents (Renal®) or flavonoids and probiotics (Renal advanced®), compared to placebo, reduce mortality rate due to uremic crises in dogs with spontaneous CKD, fed a renal diet.

Design—Masked, randomized, controlled clinical trial.

Animals—43 dogs with spontaneous CKD in IRIS stages ≥ 2, fed a renal diet.

Procedure—Dogs were randomly allocated to receive placebo (group A), Renal® (group B), or Renal® plus Renal advanced® (group C). During a first 4-week period all dogs were fed a renal diet and then randomized and clinically evaluated up to 44 weeks. The effects of dietary supplements on mortality rate due to uremic crises were assessed.

Results—Compared to group A, dogs in group B and C had approximately 50% lower mortality rate due to uremic crises (p=0.038 and p=0.041, respectively); differences were not observed between group B and C. Three dogs in group B and 3 in group C had a reduction in serum urea and creatinine concentrations after 16-20 weeks of treatment.

Conclusions and Clinical Relevance—Dietary supplementation with chitosan and alkalinizing agents along with a renal diet is beneficial in reducing mortality rate in dogs with spontaneous CKD in IRIS stages ≥ 2, and reduces concentrations of serum urea and creatinine in some dogs. The dietary supplement may lessens progression of renal failure in CKD dogs.

Key words – dog, CKD, renal diet, death, uremic crisis.

Introduction

There is a consensus to use dietary modification in dogs affected by chronic kidney disease (CKD).[1-7] Feeding a renal diet (RD) in dogs with mild and moderate spontaneous CKD had beneficial effects on uremia and mortality rate compared to a maintenance diet.[1] In addition, phosphate retention and renal secondary hyperparathyroidism are common complications of CKD,[2-7] and hyperphosphatemia is associated with the development of renal lesions in dogs and cats.[2-7] In humans and cats, oral supplementation with compounds such as chitosan (produced by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans and cell wall of fungi), calcium carbonate, aluminium hydroxide, and potassium citrate has been advocated to control hyperphosphatemia and to reduce azotemia during spontaneous CKD.[4-11] Laboratory and clinical studies in cats have demonstrated a renoprotective effect of dietary phosphate restriction with chitosan.[10] However, whether this holds true for dogs has not been assessed. Aim of the present study was to evaluate the efficacy of two commercial oral supplements rich in chitosan, enteric phosphate binders and alkalinizing agents (Renal®) [a], or rich in flavonoids, probiotics, prebiotics and vitamin (Renal advanced®) [a], in reducing mortality rate related to uremic crises in dogs affected by spontaneous CKD in IRIS stages 2, 3 and 4,[4,12] fed a RD.

Materials and Methods

Animals

Dogs with CKD were recruited at the Clinica Veterinaria Pirani of Reggio Emilia, Italy. Results of history, physical examinations, including body weight (BW) and body condition score (BCS) using a 1 to 5 scoring system (3 optimal), CBC, serum biochemical profile, urinalysis, urine protein-to-creatinine (UPC) ratio, hemogasanalysis, and indirect blood pressure measurements were collected. All dogs underwent abdominal ultrasonographic examination, which was performed by the same operator (AZ) and with the same instrument[e]. Dogs of any age were included if presenting stable renal function, as defined by serum creatinine concentrations above 1.4 mg/dL (International Renal Interest Society (IRIS) Staging System ≥ 2) that did not increase or decrease by 15% or more within 4 weeks from initial determination.[4] In the first 4-week period all dogs were started on a RD[b,c]. Dogs were excluded if affected by inflammation or infection of the genitourinary tract, cardiac disease, neoplasia and endocrinopathies. As a standard, dogs with arterial pressure (AP) substage 3 of the IRIS Staging System[12] were treated with oral amlodipine at 0.1 to 0.5 mg/kg, q24h, in order to reduce AP to substage 1 or 0.[2-7,13,14] Dogs with serum albumin concentration ≤ 2.0 g/dL received oral acetylsalicylic acid at 2.0 mg/kg, q24h, to prevent thrombosis.[4-7]

Study design

A randomized, masked, controlled clinical trial was performed using a software to allocate cases.[d] Informed consent to participate in the study was signed by dog owners.

Following inclusion, all dogs started a RD[b,c]. After 4 weeksdogs were clinically re-evaluated, performing all above laboratory and instrumental analyses, and assigned to group A (placebo), or treatment groups B (Renal®) or C (Renal® plus Renal advanced®); composition of the dietary supplements is provided in Table 1.

To mask the identity of the 3 supplements, they were formulated as powders with identical colours and contained in the same package. After assignment to group A, B or C, dogs were reassessed

between week 4 and 8. Thereafter, examinations were scheduled every 4 months and up to 44 weeks of treatment, or earlier if worsening of clinical signs was noted by the owner.

Blood sampling and assay

During each examination a blood sample was collected in overnight fasted dogs, and serum was obtained within 30 minutes, stored at 4°C and analyzed within 24 hours. Hemogasanalysis[f] was immediately performed in all cases.

CBC[g] and serum biochemical analysis, including albumin, total proteins, glucose, bilirubin, cholesterol, amylase, alanine transferase, alkaline phosphatase, urea nitrogen, creatinine, ionized calcium, sodium, potassium, chloride and phosphate, were determined by use of standard methods [h]. Blood samples were labelled with alphanumeric codes assigned by randomization to ensure that laboratory personal were blinded during processing.

Urine sample and urinalysis

During abdominal ultrasonography, an echo-guided cystocentesis was performed in all dogs, by use of a 5 mL syringe connected to a 23-gauge needle. All urine samples were put in 10 mL, sterile, evacuated collection tubes labelled with alphanumeric codes based on the previous randomization. All urine samples were analyzed by the same operator (FN). Urines were examined within 60 minutes from collection if samples were stored at room temperature (approximately 20°C), or within 4 hours if samples were stored at 4° to 8°C. Urine sediment was obtained by centrifugation (10 minutes at 900 × *g*) of 5 mL of urine, followed by removal of 4.5 mL of supernatant, and by resuspension of the remaining 0.5 mL of urine. A sample of 12 µL of the resuspended urine was microscopically assessed. The supernatant was transferred into separate tubes and stored at –20°C to determine urine protein and creatinine concentration within 7 days. RBCs and WBCs were expressed as mean number of cells/10 hpf (40 × magnification). Urine sediment with bacteriuria, > 5 RBCs or WBCs/hpf, was considered indicative of active inflammation.

UPC ratio

To calculate the UPC ratio, protein concentration (mg/dL) was measured with pyrogallol red, and creatinine (mg/dL) was measured by use of the Jaffé method in undiluted urine that was thawed before the analysis[h]. Analytes were measured in an automated spectrophotometer[h]. Dogs were classified as nonproteinuric, borderline proteinuric, or proteinuric according to the IRIS Staging System (UPC ratio < 0.2 = nonproteinuric, UPC ratio 0.2 to 0.5 = borderline proteinuric, and UPC ratio > 0.5 = proteinuric).[4,12]

Blood pressure measurement

Systolic blood pressure measurements were obtained by use of an ultrasonic Doppler device[i] in all dogs.[13,14]

Diagnosis of uremic crisis

Diagnosis of uremic crisis was established by clinicians involved in patient management unaware of the supplement being administered. Uremic crisis was defined when all of the 3 following findings were observed: i) identification of at least 2 clinical signs consistent with uremia including depression, lethargy, anorexia, vomiting, uremic breath odour, or uremic stomatitis; ii) serum urea nitrogen concentration at least 20% greater than the previously determined value; and iii) no plausible alternative for these clinical signs.[1]

Establishing cause of death

Causes of death were categorized as non-renal, probably renal, or renal based on results of anamnesis, physical examination, blood and urine tests, and criteria used to define uremic crisis. To avoid bias, only dogs classified in the third category were considered to have died from a renal event (uremic crisis). Necropsies were not performed in any case.

Statistical analysis

Dog characteristics were compared at the time of group assignment and during re-examination using the Mann-Whitney nonparametric test. Kaplan-Meier followed by log rank test was used to compare rates of death due to uremic crisis between groups. In addition, the Cox proportional hazard regression model was used to evaluate the effect of oral supplementation on the relative risk (RR) of developing death related to uremic crisis. Statistical analysis was performed with a commercial software[1], using the intention-to-treat principle. Significance was defined as $p<0.05$.

Results

Dogs and groups

Forty-three dogs were enrolled in the study, with a mean age of 5 years (range: 10 months-13 years). Five dogs were intact females, 22 were spayed females, 15 were males and 1 was a castrated male. Eight dogs were mixed breed, 3 each Dalmatian and German Shepherd, 2 each American Pittbull, Beagle, Boxer, Cavalier King Charles Spaniel, Dobermann, Golden Retriever, Labrador Retriever, Rottweiler and Shih-Tzu, and one each Bernese Mountain, Border Collie, Bullmastiff, English Bulldog, English Cocker, English Setter, Greyhound, Irish Wolfhound, Miniature Poodle, York-Shire Terrier. Mean BW was 23.3 kg (range: 2.5-71.5).

Fifteen dogs were allocated in group A, 16 in group B, and 12 in group C. At the time of allocation there was no statistical difference between the groups with regard to signalment, physical examination findings, and hematological or serum biochemical results. Serum creatinine concentration of dogs according to the IRIS Staging System is reported in Table 2.

Follow-up concentrations of creatinine, urea, phosphate, calcium, potassium, bicarbonate, and results of hemogasanalysis

Mean serum concentrations of creatinine, urea, phosphate and bicarbonate, and results of hemogasanalysis were all improved in groups B and C when values recorded at 4-8 weeks following supplement administration were compared with those collected at randomization. Potassium and calcium remained stable in both group B and C, and no episodes of hyperkalemia or of hypercalcemia was identified during the study period. There was a significant increase in BW and BCS in group B and C 4-8 weeks following enrolment (p <0.05). All other parameters included in the analysis were not different between groups. Of note, 3 dogs in group B and 3 in group C experienced a reduction of serum urea and creatinine concentrations after 16-20 weeks of treatment (Figure 1), and maintained stable values at the end of the study; in these dogs body weight did not decrease.

174

175 *Association between dietary supplements and death*

176 Compared to group A, the use of oral supplements decreased chance of death in both group B
177 (p=0.038) and C (p=0.041). By the end of the study, 9 out of 15 (60%) dogs in group A were dead,
178 against 4 out of 16 (25%) in group B and 4 out of 12 (33%) in group C (Table 2); chance of death
179 was not different between group B and C (p=0.240).

180 Compared to group A, survival of dogs in group B or in group C was not different. However,
181 survival of dogs in group B combined with those of group C was significantly longer than in control
182 dogs (p = 0.042; Logrank test p = 0.008; RR = 0.472; 95%CI: 0.23 – 0.98) (Figure 2). By the end of
183 the study, 20 out of 28 (72%) dogs in the combined B and C treatment group were alive, compared
184 to 6 out of 15 (40%) dogs in the control group (Table 2).

185 Non-renal causes of death included one case of aspiration pneumonia in the treatment group B.
186 None dog died due to non-renal causes in group C. In group A, one dog died because of
187 disseminated hemangiosarcoma. The percentage of deaths due to non-renal causes did not differ
188 between groups.

189

Discussion

In a study, admission concentration of serum creatinine did not influence survival in dogs with spontaneous CKD if a RD was administered.[1] Indeed, median survival for 21 dogs with a mean serum creatinine concentration of 3.3 mg/dL was 615 days, and median survival for dogs with serum creatinine between 2.0 and 3.1 mg/dL was also 615 days. Differently, among 17 dogs fed a maintenance diet, median survival for dogs with a mean serum creatinine of 3.7 mg/dL was of only 252 days, whereas that of the subpopulation with serum creatinine between 2.0 and 3.1 mg/dL was 461 days.

In the present investigation all dogs were fed a RD, and the mean serum creatinine concentration at the time of randomization was 5.9, 4.9 and 5.0 mg/dL, in group A, B and C, respectively. Twelve out of 16 (75%) dogs in group B, and 8 out of 12 (66.6%) in group C were alive after 336 days, compared to 6 out of 15 (40%) dogs in the group A, suggesting that chitosan together with alkalinizing agents are useful in maintaining long-term good clinical conditions in dogs, similar to previous studies performed in humans and cats.[8-11] The non significant difference observed between dog groups B and C might be related to the limited period of observation or to a real lack of benefit of flavonoids, prebiotics and probiotics on renal function in this species. Results of our series emphasize the efficacy of adding a combination of chitosan, enteric phosphate binders and alkalinizing agents to RD in reducing death for uremic crises in dogs affected by spontaneous CKD and azotemic based on the IRIS Staging System (stages ≥ 2; p=0.043).

In addition, of special interest is the reduction in serum creatinine concentration observed after 16-20 weeks of treatment in 6 dogs, 3 each allocated in group B and C (Figure 1). In dogs and cats affected by CKD, reduction in serum creatinine level is frequently related to hypomiotrophia and BCS worsening: 6 dogs in the study, in spite of stable or improved BCS respect to initial values, showed reduction of serum creatinine concentrations. In humans, two possible explanations have been proposed for the reduction of serum concentrations of nitrogen metabolites, including the increased clearance of nitrogen metabolites due to compensatory hypertrophy of the remaining

nephrons, and the enhanced excretion of chitosan bound to nitrogen metabolites in the digestive tract.[8,15] Furthermore, it has been reported that chitosan can combine with acidic substances suspected to be uremic toxins, resulting in their excretion from the body and in clinical improvementconcentrations.[8,16]

In summary, the present study shows a beneficial effect of a commercial combination of chitosan, enteric phosphate binders and alkalinizing agents in dogs affected by spontaneous CKD, fed a RD. The fact that serum urea, creatinine and inorganic phosphate concentrations, and bicarbonate improved is consistent with the hypothesis that delay in development of uremic crises and associated mortality rate was related, at least in part, with a reduction in the progression rate of renal failure.

Notes

[a] Renal® and Renal advanced® - Istituto Farmaceutico Candioli SpA, Italy.

[b] Hill's Prescription Diet Canine k/d®, Hill's Pet Nutrition Inc, Topeka, Kan.

[c] Royal Canin Renal Canine®, Royal Canin SA, Aimargues, France.

[d] GraphPad QuickCalcs calculator by GraphPad Software, Inc (2002-2005).

[e] Philips HD11XE or Philips HD7XE, Philips Ultrasound, Bothell, Washington, USA.

[f] Rapidpoint® 400, Bayer Health Care, Tarrytown (NY), USA.

[g] BC-2800Vet, MINDRAY, Mindray Co., Ltd. Shenzhen, China.

[h] Cobas Mira, Roche Diagnostic, Basel, Switzerland.

[i] DOP 2001, SAMED Elettromedicali srl, Merlino (LO), Italy.

[l] MedCalc®, Version 11.3.0.0.

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